Further Branching of Valproate-Related Carboxylic Acids Reduces the Teratogenic Activity, but Not the Anticonvulsant Effect

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Received December 29, 1995⊗

In the present study, compounds derived from the anticonvulsant drug valproic acid (VPA, 2-*n*-propylpentanoic acid) and analogues known to be teratogenic were synthesized with an additional carbon-branching in one of the side chains. The substances were tested for their ability to induce anticonvulsant activity and sedation in adult mice, and neural tube defects (exencephaly) in the offspring of pregnant animals (Han:NMRI mice). In all cases, the rates of exencephaly, embryolethality, and fetal weight retardation induced by the methyl-branched derivatives were very low when compared to those of the parent compounds. These novel compounds exhibited anticonvulsant activity which was not significantly different from that of VPA. Neurotoxicity was considerably lower for some compounds as compared to VPA. Anticonvulsant activity and neurotoxicity of branched short chain fatty acids are far less structure-dependent and not related to teratogenic potency. Within this series of compounds, (±)-4-methyl-2-*n*-propyl-4-pentenoic acid and (±)-2-isobutyl-4-pentenoic acid exhibited the most favorable profile in regard to high anticonvulsant effect, low sedation, and teratogenicity. Valproic acid analogues with additional methyl branching may be valuable antiepileptic agents with low teratogenic potential.

Introduction

After the discovery by Meunier et al. (*1*) of the anticonvulsant properties of valproic acid (2-*n*-propylpentanoic acid, VPA),[1] the drug quickly found widespread use as a valuable therapeutic agent in the treatment of several forms of epilepsy. Although originally considered to be of low toxicity, VPA unfortunately proved to have considerable teratogenic potential in the human. The most apparent and severe teratogenic effect observed is spina bifida, a posterior neural tube defect (*2*). VPA is also teratogenic in a variety of experimental animals (*3*, *4*). In mice, posterior (*5*) as well as anterior (*3*, *4*) neural tube defects (exencephaly and spina bifida, respectively) can be induced in the offspring depending on the time and frequency of application during pregnancy. Experimental studies in vivo and in vitro suggest that the parent drug VPA is very likely the proximate teratogen (*6*); metabolites play a minor role in this regard because teratogenic metabolites such as 4-en-VPA (see below) are present at low concentrations only.

Studies with a number of analogues and metabolites of VPA indicate that the teratogenic potential is strictly related to the structure of the administered compound: VPA, its metabolite 4-en-VPA [(±)-2-*n*-propyl-4-pentenoic acid] (*7*), and several analogues, i.e., (±)-2-ethylpentanoic acid (*7*), (±)-2-ethylhexanoic acid (*8*, *9*), (±)-2-butylhexanoic acid (*7*), and 4-yn-VPA [(±)-2-*n*-propyl-4-pentynoic acid] (*10*), were teratogenic, while many structural analogues of VPA such as (*E*)-2-*n*-propyl-2-pentenoic acid [(*E*)-2-en-VPA] exhibited low, if any teratogenic potential (*7*, *11*). These studies suggest that several structural elements are required for the expression of teratogenicity by VPA congeners: (a) a free carboxylic group; valpromide, the amide of VPA, is not teratogenic; (b) branching on carbon-2; (c) presence of a hydrogen atom on carbon-2; additional alkylation at carbon-2 [1-methyl-1-cyclohexanoic acid; (±)-2-methyl-2-ethylhexanoic acid; 2-methyl-VPA] or unsaturation on carbon-2 [(*E*)-2-en-VPA] abolishes teratogenicity.

But the structure specificity goes even further. In studies examining the enantiomers of chiral VPA analogues, it was found that the teratogenic potential is enantioselective: the pairs of enantiomers tested exhibited differing teratogenic potencies, but similar pharmacokinetic profiles and embryonic exposure (*8*−*12*). For example, (*S*)-4-yn-VPA proved to be 8 times more potent than the *R*-enantiomer in regard to the induction of exencephaly in mice (*10*). The high stereoselectivity of the teratogenic response was also observed in whole rodent embryo cultures (*13*). In contrast, other pharmacodynamic end points such as neurotoxicity and anticonvulsant activity did not show such a strict structure dependence: these activities were related more to lipophilicity and volume rather than their stereochemistry (*11*, *14*, *15*). Consequently, pairs of enantiomers had comparable neurotoxic and anticonvulsant properties.

This strict stereochemical requirement of teratogenic action called for further investigation aimed at elucidating the structural elements in the molecule of VPA-type carboxylic acids which are related to teratogenic potency. Such structural information may also be used in studies of the mechanism of teratogenic action of VPA-type carboxylic acids (*16*, *17*). The present study was based on the observation that compound **1b** (Scheme 1), which

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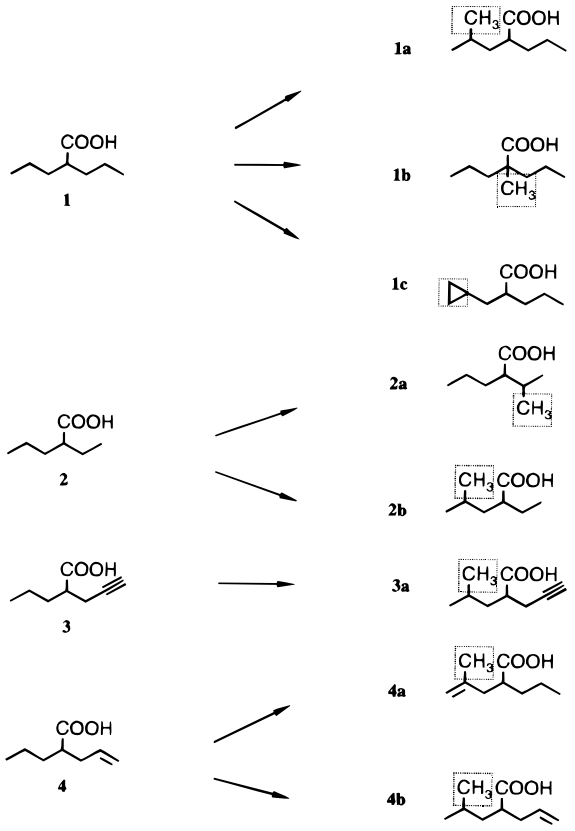
⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1996.

[1] Abbreviations: VPA, valproic acid, 2-*n*-propylpentanoic acid; 4-en-VPA, (±)-2-*n*-propyl-4-pentenoic acid; 4-yn-VPA, (±)-2-*n*-propyl-4-pentynoic acid; PTZ, pentylenetetrazole.

S0893-228x(95)00216-5 CCC: $12.00

Scheme 1.
Teratogenic Compounds (Left) and Congeners with Low Teratogenic Potency (Right)



possesses an additional branching compared to VPA, was not teratogenic (*11*). To test the hypothesis that the methyl group was responsible for the observed loss of teratogenic potency, we synthesized further methyl derivatives of VPA and other teratogenic carboxylic acids (Scheme 1). The following pharmacological properties were determined: teratogenicity as exencephaly, embryotoxicity (embryolethality and weight retardation), neurotoxicity, and anticonvulsant activity.

Materials and Methods

Chemistry. Melting points were determined using a Dr. Tottoli (Büchi, Switzerland) capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Bruker AC 250-MHz spectrometer using tetramethylsilane as an internal standard. Chemical purity was assessed by titration with standardized 1 N NaOH (Merck, Germany) and by GC/MS. GC/MS was performed by previously published methods (*18*) with some modifications: A Hewlett Packard system (type HP 5890A gas chromatograph, type 5971 MSD mass spectrometer operated by a MS Chem Station). Compounds of interest were dissolved in acetonitrile and treated with MSTFA [*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide] (Pierce, Bender & Hobein GmbH, München, Germany), 2 h, room temperature. GC separations were achieved using a 50 m × 0.2 mm i.d. HP-1 capillary column (0.11 μm film thickness) with helium as carrier gas (0.5 mL/min). The initial temperature of 70 °C was held for 1 min, and then raised by 10 °C/min to 170 °C. The injector temperature was 250 °C. The mass spectrometer (electron impact) was operated in scan mode (*m/z*: 60−270). The relative intensities of the various ions were related to the TMS ion (*m/z* = 73). Thin layer chromatography (TLC) was performed using silica gel plates (Merck, Kieselgel 60_F-254) with *n*-hexane/ethyl acetate = 7/1 plus 5% acetic acid (v/v) as the mobile phase. After drying, the plates were sprayed with 50% sulfuric acid and heated for 15 min at 150 °C. VPA analogues and precursors appeared as dark spots. Preparative column chromatography was performed with silica gel (Merck, 0.040−0.063 mm, 230−400 mesh) by flash chromatography (*19*) with eluents as described for TLC.

Dialkylated malonic acid diethyl esters were prepared by three different synthetic procedures (all reaction vessels had to be dried by heating and were flushed with argon or nitrogen during reactions):

(a) Sodium (0.1 mol) was dissolved in 50 mL of dry ethanol. Malonic acid diethyl ester or monoalkylmalonic acid diethyl ester (0.1 mol) diluted with 20 mL of ethanol was added slowly while stirring to the hot solution. Alkyl halide (0.12 mol) was added in such a way as to keep the mixture at a steady boil. After the addition of the alkyl halide was completed, the mixture was kept boiling by external heating for 2−8 h until TLC showed no starting material. Ethanol was distilled off and water added to dissolve salts. The mixture was extracted three times with ether and dried over anhydrous sodium sulfate. Evaporation of the combined ether extracts yielded crude product.

(b) To a stirred mixture of 0.1 mol of malonic ester (or monoalkyl derivative) and 0.12 mol of alkyl halide was added a solution of 0.1 mol of sodium in 50 mL of dry ethanol to keep the mixture at a slow boil. The mixture was heated until TLC indicated completion of reaction. Workup was carried out as described for (a).

(c) A suspension of 0.1 mol of sodium hydride in 35 mL of dry dimethylformamide was prepared, and monoalkylmalonic ester was added slowly such that the temperature remained below 50 °C. After hydrogen gas evolution subsided, the mixture was stirred for another 15 min. Alkyl halide (0.12 mol) was added very slowly such that the temperature remained below 40 °C. After addition was completed, the mixture was stirred at room temperature for 2 h. The mixture was poured into 300 mL of water and extracted three times with ether. Drying over anhydrous sodium sulfate and evaporation of solute afforded crude product.

Products gained from procedures a−c were distilled under reduced pressure. The dialkylated malonic esters were heated in a solution of 20.3 g (0.35 mol) of potassium hydroxide, 50 mL of water, and 100 mL of ethanol (5−12 h). After saponification was completed, ethanol was evaporated. The remaining residue was diluted with water and washed with ether. The water layer was acidified with concentrated HCl (pH <2) and extracted with ether. Drying over anhydrous sodium sulfate and concentration under reduced pressure yielded crude dialkylmalonic acid. Decarboxylation was achieved by heating of the crude product (120−180 °C). The dark residue was distilled twice *in vacuo* or alternatively chromatographed and distilled to afford the desired products:

(±)-2-Isobutylpentanoic acid (1a) (*20*) was prepared by method a, with isobutyl bromide: yield 42%; bp 75−77 °C (0.6 mbar) [lit. bp 122 °C (11 mbar)]; ^{1}H-NMR ($CDCl_3$): δ = 0.84 (9H, m, 3 × CH_3), 1.36 (4H, m_c, 3_a-CH_2, $1'_a$-CH_2, 4-CH_2), 1.62 (3H, m_c, 3_b-CH_2, $1'_b$-CH_2, 2′-CH), 2.48 (1H, m, 2-CH), 11.24 (1H, s, broad, COOH); *m/z* = 215 (54, $M^+ - CH_3$). Anal. ($C_9H_{18}O_2$).

2-Methyl-2-*n*-propylpentanoic acid (1b) (*21*). 2-*n*-Propylpentanoic acid (11 mL, 70 mmol) was added dropwise to a stirred solution of 150 mmol of lithium diisopropylamide in 100 mL of tetrahydrofuran. To complete metalization, the mixture was heated to 50 °C. The solution was allowed to cool to room temperature, and 70 mmol of methyl iodide was added. Stirring was continued for 2 h, after which the solution was acidified with 200 mL of 3 M hydrochloric acid and extracted with *n*-hexane. Drying over anhydrous sodium sulfate and distillation afforded 3.5 g (32%) of **1b**: bp 71 °C (0.5 mbar) [lit. bp 118−122 °C (20 mbar)]; ^{1}H-NMR ($CDCl_3$): δ = 0.91 (6H, t, *J* = 7 Hz, 5-CH_3, 3′-CH_3), 1.15 (3H, s, 2-CH_3), 1.19−1.44 and 1.56−1.68 (8H, 2m, 3-CH_2, 4-CH_2, 1′-CH_2, 2′-CH_2), 12.12 (1H, s, broad, COOH); *m/z* = 215 (36, $M^+ - CH_3$), 188 (22, $M^+ - C_3H_6$). Anal. ($C_9H_{18}O_2$).

(±)-2-(Cyclopropylmethyl)pentanoic acid (1c) was prepared by method a, with chloromethylcyclopropane: yield 48%; bp 91−93 °C (1.2 mbar); ^{1}H-NMR ($CDCl_3$): δ = −0.08−0.12 (2H, m, C_3H_5-H_{trans}), 0.28−0.48 (2H, m, C_3H_5-H_{cis}), 0.61−0.76 (1H,

m, 2′-CH), 0.89 (3H, t, J = 8 Hz, 5-CH_3), 1.21−1.68 (6H, m, 3-CH_2, 4-CH_2, 1′-CH_2), 2.46 (1H, m, 2-CH), 12.04 (1H, s, broad, COOH); m/z = 213 (30, $M^+ - CH_3$), 199 (89, $M^+ - C_2H_5$). Anal. ($C_9H_{16}O_2$).

(±)-2-Isopropylpentanoic acid (2a) (*20*) was prepared by method c, with isopropyl bromide: yield 44%; bp 170 °C (55 mbar) [lit. bp 112−113 (12 mbar)]; ^{1}H-NMR ($CDCl_3$): δ = 0.90 (9H, m, 3 × CH_3), 1.40 (4H, m_c, 3-CH_2, 4-CH_2), 1.85 (1H, m, 1′-CH), 2.10 (1H, m, 2-CH), 12.20 (1H, s, broad, COOH); m/z = 201 (78, $M^+ - CH_3$). Anal. ($C_8H_{16}O_2$).

(±)-2-Ethyl-4-methylpentanoic acid (2b) (*22*) was prepared by method c, using isobutyl bromide: yield 45%; bp 114−115 °C (1 mbar); ^{1}H-NMR ($CDCl_3$): δ = 0.90 (9H, m, 3 × CH_3), 1.30 (2H, m, 3_a-CH_2, $1'_a$-CH_2), 1.65 (3H, m, 3_b-CH_2, $1'_b$-CH_2, 4-CH), 2.40 (1H, m, 2-CH), 11.93 (1H, s, broad, COOH); m/z = 201 (60, $M^+ - CH_3$). Anal. ($C_8H_{16}O_2$).

(±)-2-*n*-Propyl-4-pentynoic acid (3) (*23*) was prepared by method a, with propagyl chloride: yield 44%; bp 75−76 °C (0.7 mbar) [lit. bp 82−83 °C (2.6 mbar)]; ^{1}H-NMR ($CDCl_3$): δ = 0.94 (3H, t, J = 7.5 Hz, 3′-CH_3), 1.28−1.50 (2H, m, 2′-CH_2), 1.57−1.81 (2H, m, 1′-CH_2), 2.02 (1H, t, $J \approx$ 2.5 Hz, 5-CH), 2.35−2.53 (2H, m, 3-CH_2), 2.62 (1H, m, 2-CH), 12.00 (1H, s, broad, COOH); m/z = 197 (32, $M^+ - CH_3$), 170 (20, $M^+ - C_3H_6$). Anal. ($C_8H_{12}O_2$).

(±)-2-Isobutyl-4-pentynoic acid (3a) was prepared from isobutylmalonic acid diethyl ester and 3-bromopropyne by method b: yield 40%; bp 61−62 °C (0.1 mbar); ^{1}H-NMR ($CDCl_3$): δ = 0.92 (6H, 2d, $J \approx$ 5 Hz, 2 × CH_3), 1.48 (1H, m, 2′-CH), 1.68 (2H, m, 1′-CH_2), 2.04 (1H, t, $J \approx$ 2 Hz, 5-CH), 2.48 (2H, m, 3-CH_2), 2.68 (1H, m, 2-CH), 11.82 (1H, s, broad, COOH); m/z = 211 (35, $M^+ - CH_3$). Anal. ($C_9H_{14}O_2$).

(±)-2-*n*-Propyl-4-pentenoic acid (4) (*24*) was prepared by method a, with allyl bromide: yield 49%; bp 87−88 °C (2.4 mbar); ^{1}H-NMR ($CDCl_3$): δ = 0.91 (3H, t, J = 8 Hz, 3′-CH_3), 1.30−1.73 (4H, m, 1′-CH_2, 2′-CH_2), 2.20−2.54 (3H, m, 2-CH, 3-CH_2), 5.02−5.15 (2H, m, 5-C=CH_2), 5.80 (1H, m, 4-CH=C), 12.05 (1H, s, broad, COOH); m/z = 199 (37, $M^+ - CH_3$, 185, $M^+ - C_2H_5$), 172 (29, $M^+ - C_3H_6$). Anal. ($C_8H_{14}O_2$).

(±)-4-Methyl-2-*n*-propyl-4-pentenoic acid (4a) was prepared by method a, with 3-chloro-2-methylpropene. The product was chromatographed to yield 41% pure **4a**: 70−71 °C (0.1 mbar); ^{1}H-NMR ($CDCl_3$): δ = 0.88 (3H, t, J = 7 Hz, 3′-CH_3), 1.20−1.65 (4H, m, 1′-CH_2, 2′-CH_2), 1.70 (3H, s, 4-CH_3), 2.07−2.20, 2.28−2.40 (2H, 2m, 3-CH_2), 2.56 (1H, m, 2-CH), 4.72 (2H, 2d, $J \approx$ 2 Hz, 5-C=CH_2), 11.48 (1H, s, broad, COOH); m/z = 213 (18, $M^+ - CH_3$), 199 (4, $M^+ - C_2H_5$). Anal. ($C_9H_{16}O_2$).

(±)-2-Isobutyl-4-pentenoic acid (4b) (*25*) was prepared from allylmalonic acid diethyl ester and isobutyl bromide by method b: yield 44%; bp 64−66 °C (0.7 mbar); ^{1}H-NMR ($CDCl_3$): δ = 0.88 (6H, 2d, $J \approx$ 5 Hz, 2 × CH_3), 1.28 (1H, m, 2′-CH), 1.60 (2H, m, 1′-CH_2), 2.28 (2H, m, 3-CH_2), 2.52 (1H, m, 2-CH), 5.04 (2H, m, 5-C=CH_2), 5.76 (1H, m, 4-CH=C), 9.5 (1H, s, broad, COOH); m/z = 213 (43, $M^+ - CH_3$). Anal. ($C_9H_{16}O_2$).

Other Compounds. 2-*n*-Propylpentanoic acid (VPA) (1) was purchased from Sigma (Germany). **(±)-2-*n*-Ethylpentanoic acid (2)** was provided by Desitin (Germany).

Determination of R_M Values. Substances were dissolved in acetone (20 μL/10 mL of acetone). Retention of the compounds was determined for varying developing mixtures by applying 5 μL of each solution on RP_{18}-alu sheets (Merck) 20 × 20 cm in diameter. Starting points have been positioned 3 cm from the bottom edge, at least 2.5 cm from the side with 1 cm mutual distance. Acetonitrile was used as developing organic solvent in 10/90 to 50/50 mixtures with 0.1 N phosphate buffer (pH 2.1). Runs were performed with 8 different mixtures. Detection was carried out by spraying the plates with 50% sulfuric acid and heating at 250 °C until the substances appeared as dark spots. R_M values were calculated: $R_M = \log(1/R_F - 1)$.

Biological Assays. We previously reported the animal experiments used to induce and evaluate exencephaly and embryotoxicity (*7*) as well as anticonvulsant activity and neurotoxicity (*14*).

Table 1. Teratogenicity of VPA and Analogues

substance	dose (mmol/kg) ip	live fetuses (n)	fetal weight (g)	embryolethality (%)[a]	exencephaly (%)[b]
1	3.00	122	1.07 ± 0.10	49	42
1a	3.00	79	1.24 ± 0.07[d]	3[e]	1[c]
1b	3.00	58	1.09 ± 0.12	27[e]	2[c]
1c	3.00	108	1.13 ± 0.07[d]	18[e]	5[c]
2	3.90 sc	168	1.10 ± 0.06	3	5
2a	3.00	40	1.19 ± 0.10[d]	18[e]	0
2b	3.00	46	1.23 ± 0.07[d]	4	0
3	2.47	13	0.86 ± 0.07	80	92
3a	3.00	69	1.19 ± 0.06[d]	9[e]	3[c]
4	3.00	115	1.07 ± 0.10	17	35
4a	3.00	128	1.15 ± 0.08[d]	12	1[c]
4b	3.00	86	1.20 ± 0.09[d]	6	0[c]
controls					
NaCl	3.00	126	1.14 ± 0.05	6	0

[a] Resorptions and dead fetuses as percentage of total implants. [b] Percentage of live fetuses. [c] $p < 0.01$ using χ^2 (*26*) compared to the corresponding parent compounds **1**,**3**,**4**. [d] Significantly different from respective parent compound at $p < 0.05$ using Student's t-test (*26*). [e] $p < 0.01$ using χ^2 (*26*) compared to the corresponding parent compound.

Results

The structures of the compounds tested are given in Scheme 1. Chiral compounds were used as their racemic mixtures. Compound **1c** is a rigid analogue of **1a** in that the two terminal CH_3 groups of **1a** are connected to yield a cyclopropyl moiety; the molecular volume of the cyclopropyl group is slightly less than that of the terminal dimethyl moiety of **1a** and represents an "in-between case". With the exception of **2a**, compounds with an extra methyl group in position 3 were not included in this study because such a substitution would lead to a second chiral center.

All compounds with additional methyl-branching produced exencephaly in our mouse model at rates much lower than those produced by the parent compounds **1**−**4** (Table 1). Except for compound **2** and its derivatives, these potency differences were highly significant ($p < 0.01$, χ^2 test (*26*)). Compound **1c** exhibited a higher teratogenic potency as compared to compounds **1a** and **1b**, which is compatible with the lower steric influence of the cyclopropyl methylene group as compared to the two terminal methyl groups.

Overall embryotoxicity (embryolethality, fetal weight retardation) of the methyl-branched derivatives was lower than that of the parent compounds. A tendency toward lower fetal weight retardation was particularly pronounced when the methyl-branching was located in a saturated side chain in position 4 (relative to the carboxylic acid function). The same holds true for the rate of embryolethality: e.g., compound **3a** induced significantly lower embryolethality than compound **3**. Positions for methyl-branching other than carbon 4 are less favorable in regard to obtaining compounds with low embryotoxicity, as can be seen for **2a**, **1b**, and **4a** (Table 1). It is unclear whether the embryotoxic activity is due to the same mechanism as the occurrence of exencephaly; in most cases a reduction of exencephaly rates is accompanied by a decrease in embryolethality and an increase of fetal weight. The same observation was previously made for the enantiomers of **3** (*10*).

Table 2. Anticonvulsant and Neurotoxic Activities of All Compounds Tested 15 min after ip Administration of 1.5 mmol of the Sodium Salt of the Compound/kg Body wt

substance	sc PTZ seizure threshold test[a] (%)	rotorod toxicity test[b] (%)	log *P*[e]	R_M (acetonitrile, 80%)
1	100	33	2.720	−0.4549
1a	100	67	3.119	−0.3076
1b	100[c]	80	3.119	
1c	100	20	2.635	
2	25[d]		2.192	
2a	80	67	2.590	−0.4543
2b	100	33	2.590	−0.4771
3	38	20	1.312	−0.7884
3a	60	13	1.711	−0.6886
4	63	29	2.176	−0.5754
4a	100	0	2.575	−0.4656
4b	100	0	2.575	−0.4888

[a] Percentage protection of sc pentylenetetrazole-induced threshold seizures. [b] Percentage of mice showing neurological deficits; derivatives are not significantly different from parent compounds $p < 0.05$ using χ^2 (*26*). [c] Also 100% protection at 1.0 mmol/kg. [d] 25% of VPA in MES test (*28*). [e] Estimated using *C* log *P* (*33*).

Table 3. Median Anticonvulsant Dose, Relative Potency (Compared with VPA), and Slope of the Anticonvulsant Regression Line[a]

substance	ED_{50} (mmol/kg)	rel potency	slope of regression line
1	0.71 (0.48−1.08)[b]	1	1.66 (0.96−2.74)[b]
1a	0.64 (0.41−1.01)	1.11	1.46 (0.98−2.18)
1b	0.40 (0.24−0.67)	1.75	2.10 (1.18−3.74)
1c	0.96 (0.64−1.44)	0.74	1.61 (1.02−2.54)
2a	1.12 (1.03−1.49)	0.63	1.29 (1.04−1.48)
2b	0.78 (0.52−1.16)	0.91	1.38 (1.02−1.87)
3	1.78 (1.19−2.67)	0.39	1.91 (1.02−3.59)
4	1.29 (1.00−1.67)	0.54	1.48 (1.00−2.21)
4a	0.84 (0.56−1.28)	0.85	1.45 (0.98−2.15)
4b	1.01 (0.81−1.26)	0.70	1.33 (1.10−1.61)

[a] Results are calculated according to Litchfield and Wilcoxon (*29*). [b] 95% confidence interval. Potencies and slopes of the different compounds are not significantly different from corresponding VPA values.

All substances revealed anticonvulsant activity in the subcutaneous pentylenetetrazole (PTZ) seizure threshold test, which is considered a valuable model in predicting antiepileptic activity against generalized minor seizures (petit mal, absence seizures) in the human (*27*, *28*) (Tables 2 and 3). Regression lines, calculated according to Litchfield and Wilcoxon (*29*), did not show a significant shift from that of VPA; also the slopes did not differ significantly (Table 3), indicating similar anticonvulsant potency and mode of action of this class of compounds. Anticonvulsant potency is generally increased with increasing lipophilicity (expressed as *C* log *P* values); however, there is no strict relationship between anticonvulsant activity and *C* log *P*: **4a** (*C* log *P* 2.575) was a more potent anticonvulsant than **2a** (*C* log *P* 2.590).

Neurotoxic activities of the tested compounds were recorded 15 min after ip administration (Table 2), a time period of peak neurotoxic effect (*14*) (ataxia and sedation). Within the range of the substances studied, neurotoxicity was even less related to lipophilicity than anticonvulsant activity: The more lipophilic derivatives of **3** and **4** exert lower sedative effects than their parent compounds. Furthermore, the pairs **1a** and **1b**, and **2a** and **2b**, have identical *C* log *P* values but differed in neurotoxicity. Substances with low neurotoxicity have an additional methyl group at position 4. In fact, both embryotoxicity (cf. above) and neurotoxicity were particularly low with compounds containing an additional methyl group at position 4.

As discussed above, anticonvulsant activity, neurotoxicity, and, in particular, teratogenic activity were not strictly related to lipophilicity. Substance pairs **2a**/**2b** as well as **4a**/**4b** have identical *C* log *P* values, but differ in regard to anticonvulsant and sedative properties. We have therefore tested if R_M values, determined in a partition system (RP-TLC, see Materials and Methods and Table 2), would more accurately predict the neuroactivities within the compound class studied. Determination of R_M values at 8 varying concentrations of the developer showed that compounds with identical *C* log *P* values differ slightly in their behavior in this system, while the *C* log *P* and R_M values roughly correlate. However, there was no consistent relationship between R_M values and neurotoxic and/or anticonvulsant activity.

Compounds **4a** and **4b** were selected for further evaluation because of their favorable properties such as high anticonvulsant activity and minimal side effects (neurological effects, teratogenicity). Anticonvulsant and neurotoxic activities were tested at different dose levels to determine protective indices and safety ratios (*27*). Protective indices (median neurotoxic dose, TD_{50}/median anticonvulsant dose, ED_{50}) were calculated to be 2.05 and 3.20, respectively (as compared to 2.36 for VPA), and safety ratios (TD_3/ED_{97}) 0.73 and 1.27 (VPA 0.67).

Discussion

The present study demonstrates that carboxylic acids structurally related to VPA, when substituted with an additional methyl group in positions 2, 3, or 4, exhibit greatly reduced teratogenic and embryotoxic activity, but maintain the ability to induce anticonvulsant effect. This finding extends results from earlier investigations on the very strict stereochemical requirements for VPA-type compounds to act as teratogens. Even the highly teratogenic 4-yn-VPA lost considerable potency upon introduction of a methyl group in the alkyl side chain. The interaction of the substances with the—probably chiral—biological counterpart in the embryo has to be very specific because each single structural change of any of the four groups attached to the C-2-atom results in major alteration of teratogenic potency.

Since all tested derivatives were racemic mixtures of chiral substances, their enantiomers can be expected to differ in teratogenic activity. However, the difference between the pairs of enantiomers of the methyl-substituted derivatives should not be as high as that between the pairs of enantiomers of the parent compound. This is predicted by Pfeiffer's rule (*30*) which can be restated in the present context as follows: The weaker the biological response of the racemic mixture, the smaller the difference in the toxicological effect of the optical antipodes—if the action is enantioselectively controlled. Thus, the enantioselectivity of the teratogenicity of the methyl derivatives will be relatively low.

The anticonvulsant activity and neurotoxicity are unrelated to teratogenicity and, also, do not always go hand in hand. Thus, it is possible to produce compounds with high anticonvulsant activity and low teratogenic activity as well as relatively low neurotoxicty (e.g., **4a**, **4b**). These results are compatible with the recent findings by Palaty and Abbott (*15*), who showed that the anticonvulsant potency of compounds in another set of analogues of VPA correlated with lipophilicity. We also

noted that the sedative activity is influenced more specifically by the spatial arrangements of the molecules than the anticonvulsant activity. This could mean that sedation caused by this class of compounds is not solely dictated by unspecific interactions. On the other hand, it could well be that neither log *P* values nor a partition system (R_M) always correctly represent lipophilicity in a biological system. Perhaps a correlation of anticonvulsant potency and neurotoxicity with other lipophilicity descriptors should be tried with this class of carboxylic acids. In the present series of compounds, the most favorable ratio between desirable pharmacological effect and unwanted side effect is obtained for substances with methyl-branching in position 4 and *C* log *P* values of around 2.6.

Other differences in the activities of the substances tested may be due to pharmacokinetics, a subject which is presently under investigation. Furthermore, studies on structure−activity relationships should also be carried out in regard to the hepatotoxic potential of these new analogues (*31*, *32*).

Acknowledgment. This work was supported by a grant from the Alexander von Humboldt-Stiftung to M.M.A.E., and by a grant from the BgVV Berlin (ZEBET), by American Biogenetic Sciences, and by the European Commission (Biotech Programme BIO2-CT93-0471).

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